
An improved method for photofootprinting yeast genes *in vivo* using Taq polymerase

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ABSTRACT

We have developed an improved method for photofootprinting *in vivo* which utilizes the thermostable DNA polymerase from *T. aquaticus* (Taq) in a primer extension assay. UV light is used to introduce photoproducts into the genomic DNA of intact yeast cells. The photoproducts are then detected and mapped at the nucleotide level by multiple rounds of annealing and extension using Taq polymerase, which is blocked by photoproducts in the template DNA. The method is more rapid, sensitive, and reproducible than the previously described chemical photofootprinting procedure developed in this laboratory (Nature 325, 173-177.), and detects photoproducts with a specificity which is similar, but not identical to that of the previously described procedure. Binding of GAL4 protein to its binding sites within the GAL1-10 upstream activating sequence is demonstrated using the primer extension photofootprinting method. The primer extension assay can also be used to map DNA strand breakage generated by other footprinting methods, and to determine DNA sequence directly from the yeast genome.

INTRODUCTION

A protocol for photofootprinting yeast DNA *in vivo* has been previously reported by our laboratory(1). It is based on the principle that the sensitivity of DNA to modification by UV light is dependent on its conformation, which in turn may depend on the presence or absence of proteins bound to the DNA. Selleck and Majors(1) combined the chemical photofootprinting method of Becker and Wang(2), in which DNA is chemically cleaved at UV photoproducts, with the indirect end-labelling technique of Church and Gilbert(3). Individual photoproducts produced *in vivo* in yeast genomic DNA were mapped and quantitated from yeast cells under various growth conditions to produce a footprint of the single copy GAL1-10 regulatory region(1,4,5). While the method allowed the detection of bound regulatory proteins {see also(6)}, its utility was limited by the technical difficulty of the many steps involved, the small fraction of experiments producing adequate resolution, and the requirement for a high pressure mercury arc lamp. Here, we report a new method for photofootprinting *in vivo* which is faster and easier, consistently provides high resolution, and requires only commonly available equipment.

Elongation by several DNA polymerases is terminated at UV photoproducts in the template DNA(7-9). Furthermore, Huibregtse and Engelke have demonstrated the feasibility of sequencing single copy yeast genes using primer extension(10). We therefore reasoned that we could use a primer extension assay to detect and map *in vivo* photofootprints in the yeast genome. Sequenase(11) was used in our initial experiments with the primer extension assay. However, several potential advantages prompted us to switch to Taq polymerase(12,13), with superior results. Here, we describe the development of the assay. As a model system, we chose to look at GAL4 binding to sites within the GAL1-10 upstream activating

sequence (UAS)(14), since wild type and mutant GAL4 strains were available, and since chemical photofootprinting of this region had already been completed for comparison(1,4,5). Binding of GAL4 is demonstrated *in vivo* using Taq polymerase in a primer extension based photofootprinting protocol.

METHODS

Yeast Growth and UV Irradiation

Yeast strains YM654(α ura3-52 ade2-101 his3 Δ 200 tyr1-501 lys2-801 gal80 Δ -538)=GAL4⁺ and YM709(α ura3-52 his3 Δ 200 ade2-101 lys2-801 trp1 Δ tyr1- met- can^R gal4 Δ 542 gal80 Δ 538)=GAL4 Δ , kindly provided by Mark Johnston, were grown in YP medium with the appropriate carbon source (2% glucose for YM709 or 2% raffinose for YM654) to a density of 5×10^7 cells/ml. Cells from 100ml of culture were pelleted, and irradiated when appropriate, or taken directly for DNA isolation. Irradiations were done by first suspending the cell pellet in 2.5ml PBS (0.2M NaCl, 2.7mM KCl, 15.3mM Na₂HPO₄, 1.5mM KH₂PO₄, 0.7mM CaCl₂, 0.5mM MgCl₂) containing the appropriate carbon source. For arc lamp irradiations, the cells were placed in a quartz cuvette in 250 μ l aliquots and irradiated for 60sec. in the focussed, water filtered beam of a 200W mercury arc lamp. Aliquots were pooled on ice until ready for DNA isolation. Germicidal lamp irradiations were performed by placing the suspended cells in a 100mm petri dish 4cm beneath an inverted Fotodyne model P-3-3000-254 transilluminator from which the glass filter had been removed. The transilluminator contains 4 G.E. 15W germicidal lamps. After irradiation for the indicated time, the cells were collected with several rinses of PBS, pelleted, and DNA was isolated. Naked DNA was irradiated after preparation in an uncapped microfuge tube.

DNA Isolation

DNA was isolated by a modification of the procedure of Giniger *et al.*(15) as follows. Pelleted cells from 100ml culture were resuspended in 1ml 1M sorbitol, 0.1M EDTA. 2 μ l β -mercaptoethanol and 200U lyticase were added, and cells were incubated at 37° for 30min. in a microfuge tube. Spheroplasts were pelleted for 1min., and resuspended in 1ml of 50mM Tris pH8, 20mM EDTA. The sample was divided into two tubes, 25 μ l of 20% SDS was added to each, and the spheroplasts were lysed by incubation at 68° for 30min. For irradiated samples only, 3 μ l of 20mg/ml proteinase K was added and incubated for an additional 60min. at 68°. 200 μ l 5M potassium acetate was then added and the samples incubated on ice for 60min. The samples were then pelleted for 10min. and 5min. sequentially, and the supernatants collected each time. Nucleic acids were precipitated from the resulting supernatant by addition of 0.7ml isopropanol, and pelleted for 20sec. The pellets were rinsed with 95% ethanol, and 300 μ l 10mM Tris pH8, 1mM EDTA containing 10 μ g RNaseA was added to each pellet and incubated at 37° for >2hr. The half-samples were then combined, and the DNA precipitated by addition of 3 μ l aliquots of 1M spermidine pH7 until a precipitate appeared (typically, after 2-3 aliquots). The DNA was pelleted for 20sec., the supernatant removed, and the DNA thoroughly resuspended in 300 μ l 3M ammonium acetate. The DNA was precipitated by the addition of 750 μ l ethanol, pelleted, and resuspended for digestion with HaeIII. After digestion, the DNA was phenol extracted, ethanol precipitated, and resuspended in water at a concentration of 10 μ g/7 μ l for primer extension. Typical yield was ~100 μ g/100ml cells.

Primer Preparation

The oligonucleotide used in this study was an 18mer of sequence 5' GAGCCCCATTATCTTAGC 3'

complementary to position 284 through 301 on the bottom strand of the GAL1-10 gene from the yeast *Saccharomyces cerevisiae*(16). Before use, it was purified on a DEAE column and resuspended in water at a concentration of 2.5ng/ul. 3.5ul (8.75ng) of the oligonucleotide was end-labelled with [³²P]γATP (>5000Ci/mmol), 1μg RNA was added as carrier, and the oligonucleotide was precipitated twice from ethanol. It was resuspended in 50μl water for use as a primer.

One Cycle Primer Extension

One cycle genomic primer extensions were done as follows. 10μg(7μl) of digested genomic DNA was combined with 3μl of end labelled primer (525pmol, or ~100 fold excess). For alkaline denaturation, 1μl of 2N NaOH was added. After 5min. 1.5μl 3M sodium acetate and 40μl ethanol were added. The sample was chilled on dry ice, pelleted, dried, and resuspended in the appropriate buffer. For Sequenase, that was 12.5μl of 40mM TrisHCl pH7.5, 20mM MgCl₂, 50mM NaCl, and when indicated, 5μg E. coli single strand binding protein(SSB). For Taq polymerase, that was 20μl of 50mM KCl, 10mM TrisHCl pH8.4, 2.5mM MgCl₂, 170μg/ml BSA.

For heat denaturation, the sample containing DNA and primer was increased to 20μl by addition of 10μl of 2x Taq buffer (see above), and boiled for 3min.

For Sequenase primer extension, the sample was heated at 70° for 3min., cooled at 46° for 30min., and 6U of Sequenase and all four nucleotides each at 80μM were added in a total of 10μl. For sequencing, 8μM of the appropriate dideoxynucleotide was also present. Incubation was continued at 46° for 5min. If SSB was present, 1μg proteinase K was added and incubation continued at 68° for 20min. The reaction was ended by ethanol precipitation and resuspension of the sample in 2.5μl loading buffer for sequencing gel analysis.

For Taq polymerase primer extension, the sample was heated at 95° for 3min., cooled at 46° for 10min., and 5μl of 1x Taq buffer containing 1U Taq polymerase (Perkin Elmer Cetus) and all four deoxynucleotides to a final concentration of 100μM each was added. Incubation continued at 70° for 5min. The reaction was ended by ethanol precipitation and resuspension in 2.5μl loading buffer for sequencing gel analysis.

Multiple Cycle Primer Extension

Multiple cycle primer extension was carried out as follows. 10μg genomic DNA, 3μl end labelled primer (525pmol, or ~100 fold excess), 1U Taq polymerase, 100μM final concentration of each dNTP (for sequencing, the nucleotide mixes shown in the legend to figure 6 were used), and 1x Taq buffer (see above) were combined in a total volume of 25μl. The sample was overlayed with mineral oil, and was subjected to thermal cycles of 2min. at 95°, 2min. at 46°, and 2min. at 70°. To end, the oil was drawn off, and residual oil was removed by extraction twice with chloroform. The sample was ethanol precipitated and resuspended for gel analysis as above.

Sequence markers were made by the published Sequenase protocol(17), except that the primer was the same end-labelled oligonucleotide used for footprinting, and the labelling reaction was omitted. The template was a plasmid containing the GAL1-10 intergenic region. Lanes are labelled for the template DNA.

All sequencing gels were covered with plastic wrap and exposed to Kodak XAR-5 film with an enhancing screen. All other manipulations were performed according to standard methods(18).

RESULTS

Photofootprinting with Sequenase

Initially, we desired to demonstrate that Sequenase would detect photoproducts in template DNA. Plasmid DNA bearing the GAL1-10 intergenic region as a target sequence was irradiated with a high pressure mercury arc lamp to introduce photomodifications. The DNA was then subjected to alkaline denaturation, followed by treatment identical to that used for sequencing with Sequenase(17), except that

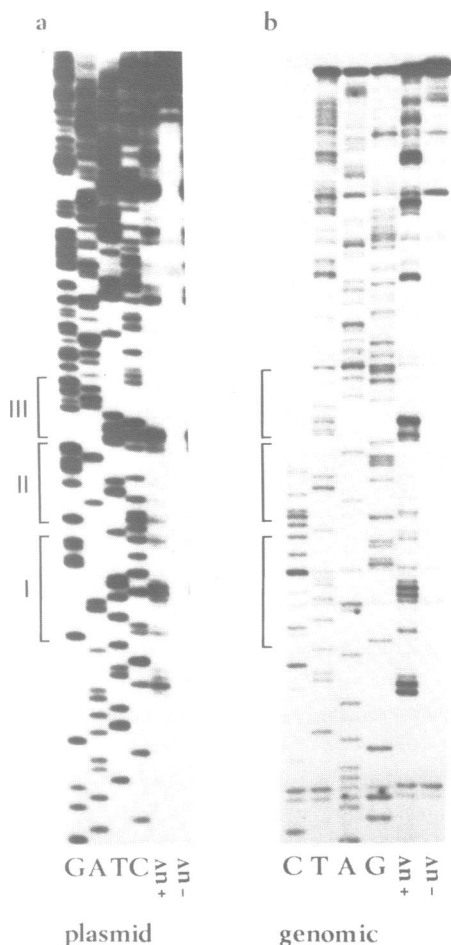


Figure 1. Photoproduct detection using Sequenase. (a) Plasmid DNA bearing the GAL1-10 UAS was sequenced using Sequenase according to the published procedure(20). 0.5 μ g of the same plasmid irradiated (+uv) for 10sec. with the arc lamp or unirradiated (-uv) was subjected to the sequencing protocol but with no dideoxynucleotides present. (b) 10 μ g genomic DNA/lane was denatured by alkali and sequenced using Sequenase. 10 μ g genomic DNA irradiated for 10sec. with the arc lamp (+uv) or unirradiated (-uv) was alkali denatured, and subjected to primer extension using Sequenase.

no dideoxynucleotides were present in the "termination" reaction. As seen in Figure 1a., numerous bands corresponding to photoproducts can be seen in the lane representing irradiated DNA that are absent in the lane representing unirradiated DNA. Thus, polymerization catalyzed by Sequenase stops at photoproducts in the template.

This method was extended to genomic DNA in a similar fashion. 10 μ g of irradiated or unirradiated genomic DNA was denatured and annealed to an end labelled oligonucleotide present at 100 fold excess. The annealed primer was then extended in the presence of Sequenase and all four deoxynucleotides. A sequencing ladder was generated by adding the appropriate dideoxynucleotide to the nucleotide mix and using unirradiated genomic DNA. The results are presented in Figure 1b. Bands present in the irradiated sample which are absent from the unirradiated sample represent photoproducts. Other experiments have shown that the temperature at which annealing and extension were carried out was critical in reducing background bands (data not shown). Even under optimal conditions, significant background can be seen (-uv, Figure 1b), which could interfere with footprint interpretation. Addition of *E. coli* single strand binding protein to the reaction was partially effective at reducing background stops (see below, Figure 2a).

Figure 2a shows an *in vivo* photofootprint of GAL4 binding sites I, II, and III, using Sequenase. Cells with wild type or deleted GAL4 were irradiated with the mercury arc lamp, DNA was isolated, and primer extension was carried out as in Figure 1b. The most dramatic footprint features seen previously in chemical photofootprinting experiments are indicated by arrows(1,4). These footprint features are reproduced, albeit weakly, by the Sequenase primer extension method. While a GAL4 footprint can be seen using this method, it is less dramatic than the chemical photofootprint(1,4) or the Taq photofootprint described below.

Photofootprinting with Taq Polymerase

Several considerations suggested the possibility that Taq polymerase might be superior to Sequenase in the footprint assay. First, the high temperatures at which Taq polymerase functions would be expected to denature any secondary structure in the template that might contribute to background. Second, Taq polymerase is stable at temperatures sufficient to denature the DNA, allowing multiple cycles of annealing and extension(13). This could amplify the specific signal strength, allowing autoradiography exposure times which are substantially shorter, and also might increase the signal to noise ratio. We therefore modified the footprint protocol to take advantage of these unique properties of Taq polymerase.

Shown in Figure 2b,c are the results of an experiment using Taq polymerase. One round of annealing at 46° and extension at 70° was performed on DNA that had been denatured either by alkali or by heat. It is apparent that Taq polymerase recognizes photoproducts with approximately the same specificity as does Sequenase (compare the presence and position of bands in a to b,c). As predicted, background bands are infrequent and weak. Remarkably, there is a dramatic enhancement in the GAL4 dependent photofootprint when detected by Taq polymerase as compared to Sequenase. We believe this enhancement results both from reduced background, and from less photoproduct readthrough with Taq polymerase. The template DNA samples in this experiment are aliquots from the same preparations.

Refinements using Taq Polymerase

To simplify and improve the procedure, we tested several modifications. First, we attempted to substitute low pressure mercury germicidal lamps for the arc lamp used previously (for a discussion of the wavelength dependence of these reactions, see refs. 19-22). Cells were suspended in PBS, evenly

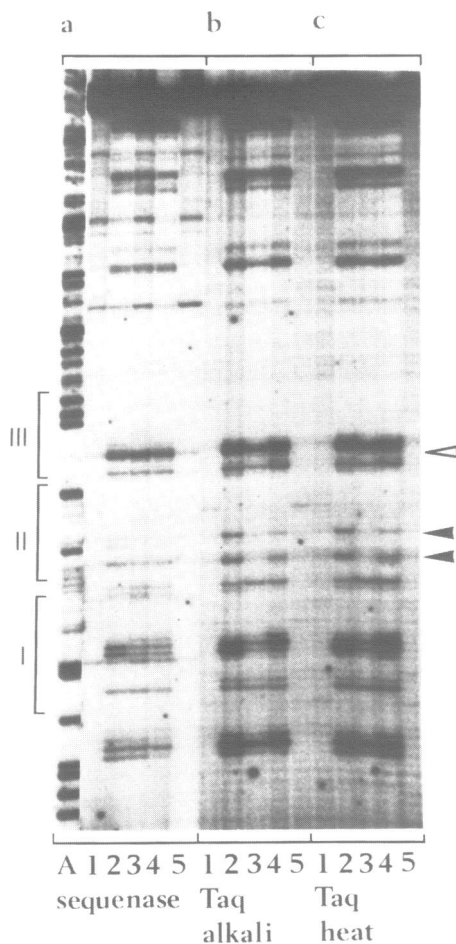


Figure 2. One cycle primer extension photofootprints using Sequenase and Taq polymerase. The samples are in each case: lanes 1 and 5, unirradiated DNA; lane 2, DNA from GAL4⁺ cells irradiated *in vivo*; lane 3, DNA from GAL4 Δ cells irradiated *in vivo*; lane 4, DNA irradiated for 10 sec. *in vitro*. (a) *in vivo* photofootprint using Sequenase plus SSB. (b and c) *in vivo* photofootprints using one cycle with Taq polymerase and alkali denaturation (b) or heat denaturation (c). Solid arrows mark GAL4 dependent enhancements; the open arrow marks a GAL4 dependent repression.

dispersed on a 100mm petri dish, and irradiated for the indicated times beneath an inverted transilluminator with the filter removed. DNA was prepared and primer extension was carried out with Taq polymerase as in Figure 2b,c. The results are shown in Figure 3. For comparison, samples irradiated with the arc lamp are also shown. Clearly, the germicidal lamps effectively photomodify the DNA *in vivo*. The transilluminator is technically much easier and faster to use than the arc lamp, since the entire sample can be irradiated in one aliquot rather than many. This irradiation method is theoretically better, since the cells are exposed to less total spectral energy which could disrupt the

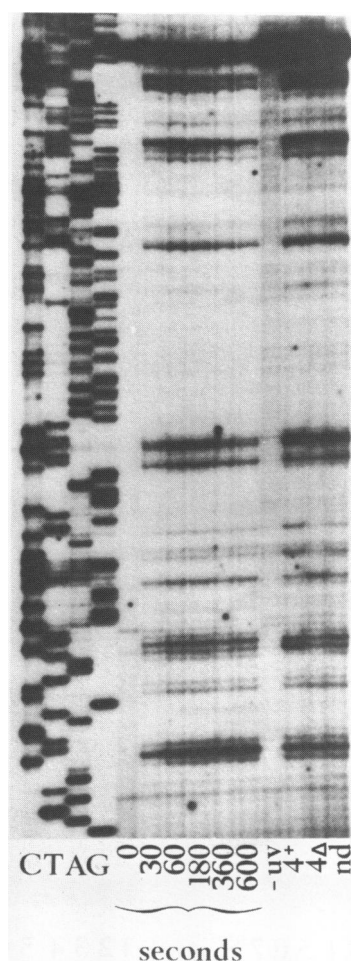


Figure 3. Time course of irradiation *in vivo* with the germicidal lamps. Cells (GAL4⁺ grown on glucose) were irradiated for the times indicated, and then subjected to one cycle primer extension with Taq polymerase. The last four lanes are identical to lanes 1-4 of Figure 2c. nd = naked DNA irradiated *in vitro*.

biological phenomena of interest. Finally, the pattern of photomodification is not highly sensitive to the irradiation time, reducing the likelihood of error from this source. A 60sec. irradiation time was chosen as optimal, and was used subsequently unless otherwise indicated.

We next tested the effects of several extension temperatures and of multiple cycles of annealing and extension (Figure 4). In lanes 1 - 4, samples were denaturated by boiling for 3min., followed by annealing at 46° for 10min. Taq polymerase and nucleotides were then added, and extension was allowed to proceed at 70°, 75° or 80° for 5min. Extension temperatures above 70° failed to produce a signal. In lanes 5 - 8, all the reaction components were combined, and the sample was cycled from 95° to 46° to

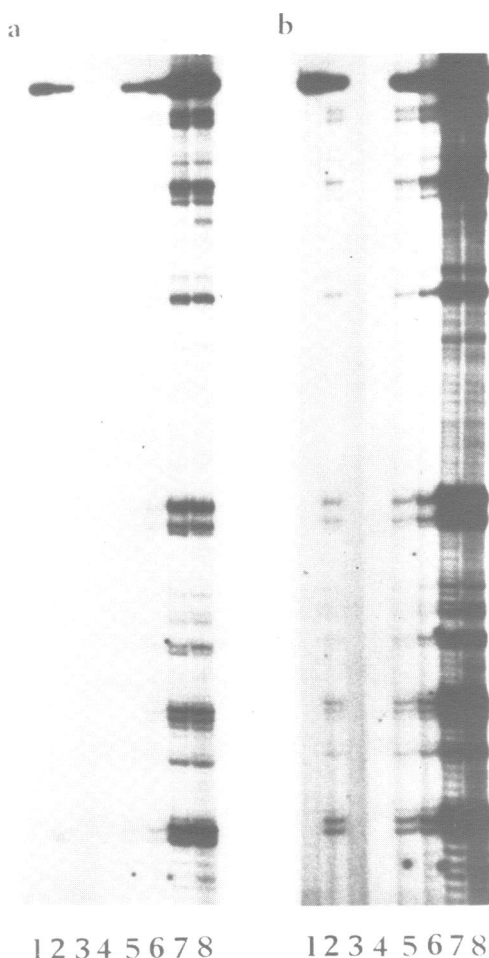


Figure 4. Temperature dependence and cycling of primer extension with Taq polymerase. Lane 1, unirradiated DNA; lanes 2-8, DNA from cells irradiated 180 sec. with the germicidal lamp. Lanes 1-4, one cycle primer extension with Taq polymerase. Denaturation was at 95° for 3min., annealing at 46° for 10min., and extension at 70° (lanes 1,2), 75° (lane 3) or 80° (lane 4) for 5min. Lanes 5-8, primer extension using Taq polymerase with denaturation at 95° for 2min., annealing at 46° for 2min., and extension at 70° for 2min. One cycle (lane 5), two cycles (lane 6), ten cycles (lane 7), and ten cycles with 300 fold excess oligo (lane 8). (a) 16 hour exposure. (b) 120 hour exposure.

70° (2min. each) for the number of cycles indicated in the figure. There is an approximately linear increase in signal strength with number of cycles over this range, which is not affected by addition of extra primer (compare lanes 7,8). The two autoradiographic exposure times show that while one cycle yields just detectable signal at 120hrs exposure, 10 cycles yields signal easily detected at 16hrs exposure. Furthermore, comparison of lane 7 of Figure 4 with those of Figure 2 or 3 shows that background is significantly reduced by using multiple cycles and a short exposure.

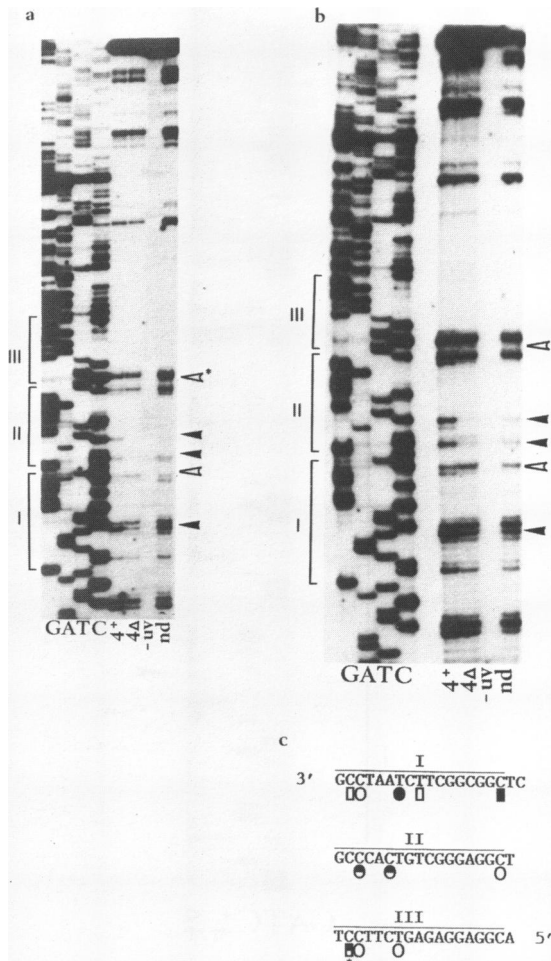


Figure 5. *In vivo* photofootprint of GAL1-10 UAS using 10 cycles with Taq polymerase. (a and b) Cells with wild type or deleted GAL4 (4⁺ or 4Δ, respectively) were irradiated *in vivo* for 60sec. using the germicidal lamps. Control DNA was irradiated (nd) for 10 sec. using the germicidal lamps, or left unirradiated (-uv). The DNA was then subjected to 10 cycles of primer extension. Enhancements are marked with solid arrows and repressions with open arrows. Only those footprint changes of which we are most confident are marked; other potential footprint changes may also be apparent. (a) and (b) show two independent experiments. (c) Summary of photofootprint changes seen with Taq polymerase (open symbols) and with chemical photofootprinting (solid symbols) in sites I, II and III. Boxes mark GAL4 dependent repressions, and circles mark GAL4 dependent enhancements. The repression in site III (*) is not easily seen in these reproductions, but was visible in every experiment (see also Figure 2). It was also a faint band in chemical photofootprinting experiments(4).

Figure 5 shows two independent *in vivo* photofootprints of the GAL4 binding sites in the absence or presence of GAL4, produced by 10 cycles of annealing and extension as described above. Comparison with the results in Figure 2 shows that the same features are visible, but that the background and the

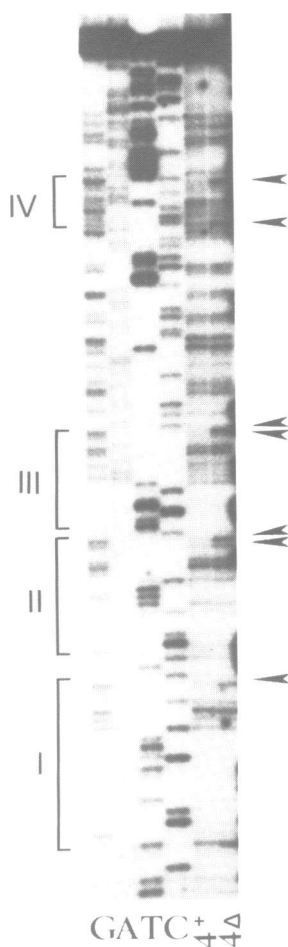


Figure 6. Genomic sequencing and dimethyl sulphate(DMS) footprinting using Taq polymerase primer extension. For sequencing, 10 cycles of primer extension were carried out on genomic DNA in the presence of the following nucleotide concentrations: C lane, 50 μ M ddGTP, 10 μ M dGTP, 100 μ M each of dATP, dCTP, and dTTP; T lane, 100 μ M ddATP, 2.5 μ M dATP, 100 μ M each of dGTP, dCTP, and dTTP; A lane, 100 μ M ddTTP, 10 μ M dTTP, 100 μ M each of dGTP, dATP, and dCTP; G lane, 100 μ M ddCTP, 10 μ M dCTP, 100 μ M each of dGTP, dATP, and dTTP. The A lane should have slightly less dTTP to be optimal for this distance from the primer. For DMS footprinting, cells bearing wild type (4⁺) or deleted GAL4 (4 Δ) were suspended in 1.5ml YP and 2 μ l DMS was added. After 2min., the reaction was quenched with 40ml cold TE (10mM Tris pH8, 1mMEDTA) containing 40mM NaCl. The cells were pelleted and DNA was isolated and cut. It was then suspended in 100 μ l 1M piperidine and heated to 90° for 30min. followed by lyophilization, which was repeated twice from 100 μ l water, ethanol precipitation twice from 100 μ l water, and a final lyophilization from 100 μ l water. 10 μ g cleaved DNA was then subjected to 10 cycle primer extension. The arrows mark GAL4 dependent repressions.

exposure time are both substantially reduced. It is interesting to compare the results from this method to those from chemical photofootprinting of the same region. A summary of the features seen by chemical photofootprinting(1,4) and by Taq polymerase is shown in Figure 5c. The strong GAL4 dependent

enhancements in binding site II and the repression in binding site III are seen with both methods. In binding site I, the primer extension assay detects a repression which was not seen using the chemical footprint method, and an enhancement where chemistry detected no photoproduct at all. Conversely, several footprint features seen using the chemical method are not detected by primer extension. At most of these positions, photoproducts are observed, but no changes are apparent. However, in binding site III, Taq polymerase fails to detect a photoproduct which was seen by the chemical detection method. This photoproduct appears to be a 5' GT 3' dimer(1,4, and unpublished data), whereas the others are dipyrimidine dimers. Thus, the specificities of the two methods differ, and the resulting footprints are somewhat different.

Other Applications

It has previously been shown that DNaseI and dimethyl sulphate/piperidine induced cleavages in plasmid DNA can be mapped using primer extension with Klenow fragment(23,24). Our genomic primer extension assay can be applied to applications other than photoproduct mapping as well. In principle, termini from any footprinting technique which cleaves DNA can be detected and mapped using this method. As a demonstration, we did a dimethyl sulphate(DMS) footprint of the GAL4 binding sites in GAL1-10. Yeast cells were exposed to DMS *in vivo* to methylate the DNA, which was then isolated, cut with HaeIII, and cleaved at methylated G residues with piperidine and heat. The DNA was then subjected to the primer extension assay as described above. The results are shown in Figure 6, and are identical to those obtained by Giniger *et al.*(15).

It is also possible to sequence genomic DNA directly using this method. In Figure 6, the sequencing lanes were produced by 10 cycles of primer extension on genomic DNA in the presence of the nucleotide mixes indicated in the figure legend in a fashion otherwise identical to the footprinting procedure.

DISCUSSION

An improved method for photofootprinting single copy yeast genes *in vivo* has been described. UV light induced photoproducts, whose formation is influenced by the presence of proteins bound to the DNA, are detected and mapped by multiple rounds of primer extension using Taq polymerase. The method is rapid and reproducible, and yields information which is different from and complementary to that derived from other footprinting procedures.

We have demonstrated the utility of this method by footprinting three of the GAL4 binding sites within the UAS of the GAL1-10 genes. GAL4 binding was detected in strains bearing GAL4 when compared to strains in which GAL4 had been deleted. The results agree with those from previous chemical photofootprinting experiments done in this laboratory which demonstrated GAL4 binding at the same sites(1,4).

The primer extension assay can be used not only to detect UV photoproducts, but to detect strand breaks of any origin. It could therefore be used for DMS, DNaseI, methidiumpropyl-EDTA-Iron(II), or S1 footprinting, for example. We have illustrated this by footprinting the GAL1-10 UAS with DMS. The resulting footprint pattern agrees precisely with that of Giniger, *et al.*(15), who detected their strand breaks by blotting and hybridization according to Church and Gilbert(3). Our method is more rapid, and has equal or greater sensitivity. In addition, Huibregtse *et al.* have recently reported the successful mapping of DNaseI cuts in yeast genomic DNA using primer extension with Sequenase(25).

It would be of interest to extend our method to use in other organisms. The limiting factor is

expected to be sensitivity, which depends on genome size. The human genome is approximately 100 fold more complex than that of yeast. In order to achieve sufficient signal, one might make several modifications to the procedure. First, one could incorporate multiple labelled nucleotides into the primer. Second, one could increase the number of cycles of annealing and extension used. Third, one could increase the exposure time of autoradiography and remain within reasonable bounds. Finally, one could enrich for the target DNA in restricted genomic samples by isolation from agarose gels. We feel that some or all of these changes should allow use of the primer extension photofootprinting method in higher eukaryotic cells.

While the primer extension and chemical photofootprinting procedures detect similar patterns of photoproducts, we have observed differences between the two methods at some sites. Some of these differences lie within the GAL1-10 UAS and have been noted above. In addition, we have found that the transcription dependent chemical photofootprints seen in the TATA element of GAL1 (1), as well as that of the SUC2 gene (unpublished observation) are not detected using Taq polymerase primer extension, although the GAL10 TATA footprint(1) is weakly detected (data not shown).

The predominant photoproducts formed in DNA exposed to 254nm UV light are the cis-syn and (6-4) dipyrimidine photodimers, although numerous other defined and undefined species also exist(22,26,27). Taq polymerase detects a number of photoproducts not detected by the chemical cleavage method, and *vice versa*. This may be attributable to the type of photoproduct, or perhaps to the sequence context in which certain photoproducts occur. The exact nature of the photoproducts formed in these experiments is unknown, so we cannot define the precise specificities of the two methods from these results. In at least one specific instance, we know that Taq polymerase is blocked by a cis-syn thymine dimer inserted synthetically into the template DNA (John-Stephen Taylor, personal communication). However, further work will be required to define more precisely the specificity of these methods. While use of the assay to detect proteins interacting with the DNA does not depend on knowing the precise nature of the underlying photoproducts, it is conceivable that determining the structure of induced or repressed photoproducts could reveal details of the particular protein DNA interaction being studied.

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